



# Digestomics of cow's milk: casein-derived digestion-resistant peptides aggregate into functional complexes

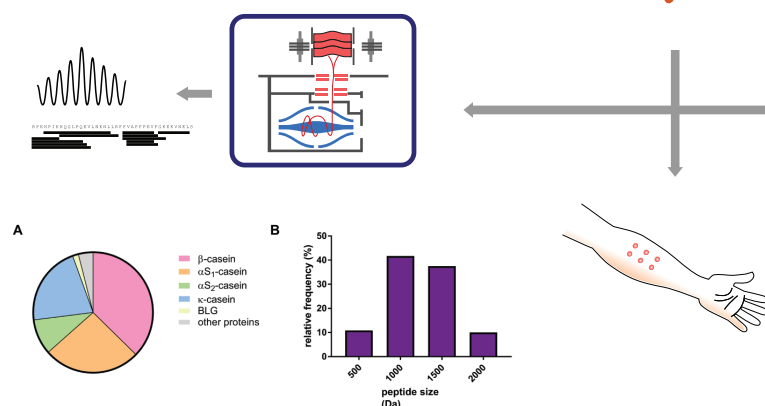
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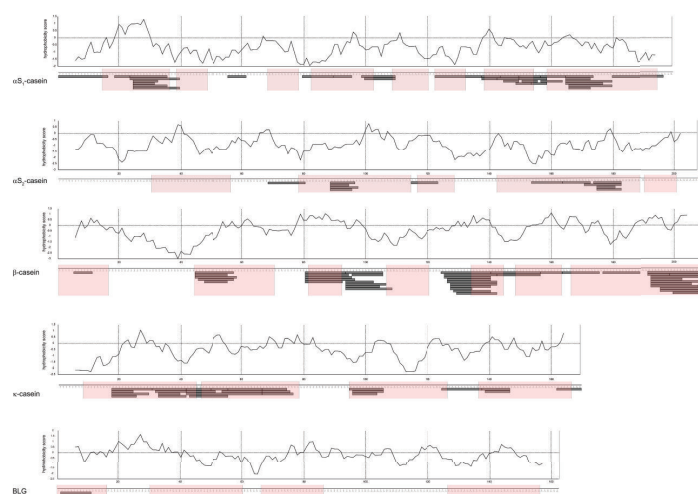
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Allergy to cow's milk proteins is commonly reported in infants, with majority of them successfully outgrowing it by the end of childhood. Safer alternatives for introduction of milk into children's diet have been investigated and milk proteins hydrolysates with fragments less than 3 kDa are considered as hypoallergenic. The aim of this study was to identify digestion products of major milk allergens and to examine the IgE reactivity and allergenicity of short digestion-resistant peptides (SDRP) released by pepsin digestion of whole milk. Here, raw milk was subjected to simulated gastric digestion and analyzed by electrophoresis and Western blotting. In gastric digests alpha-lactalbumin and beta-lactoglobulin were present mostly as intact proteins. High-resolution mass-spectrometry peptidomics analysis of SDRP fractionated from digests revealed that majority of the

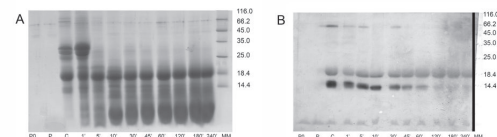
digestion-resistant peptides originated from caseins (97% of peptides). SDRP mostly overlapped with the known IgE epitopes of cow's milk allergens, with the average peptide length of  $10.6 \pm 3.5$  amino-acids. The ability of SDRP to compete for IgE binding with individual milk allergens and the mixture of the milk proteins was demonstrated. Since thirty amino acids has been suggested as a minimal length of a peptide able to cross-link two IgE molecules on the surface of mast cells and provoke an allergic reaction, it was unexpected that SDRP of induced allergic in vivo responses (positive skin-prick tests) in 4 out of 5 milk-allergic subjects. Hence, aggregation ability of the SDRP was assessed and confirmed by size-exclusion chromatography. Our results prove that short digestion-resistant peptides mainly corresponding to the continuous epitopes of milk proteins induce an allergic in-vivo response due to aggregation.



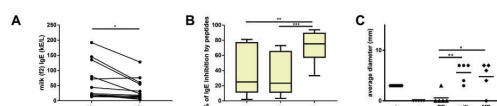
Analysis of the peptides identified after in vitro digestion of milk: A – protein origin distribution, B – peptide size distribution profile.



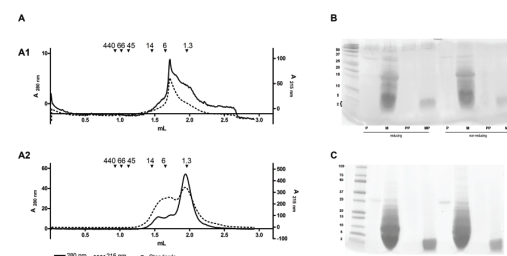
Overlap of identified digestion-resistant peptides of cow's milk proteins and hydrophobicity indexes of the proteins calculated by ProtScale web application available at ExPASy ([www.expasy.org](http://www.expasy.org)). Pink-shaded regions are known IgE-binding epitopes



Pepsin digestion of raw milk over 4-hour period: A – SDS-PAGE, B – Western blot probed with antibodies against whey proteins.



IgE-binding properties of milk-derived peptides: A – IgE binding to the milk-protein-coated solid phase (f2) in the presence of pepsin peptides (PP) or milk-derived peptides (MP), B – inhibition of IgE binding to ImmunoCAPs coated with individual milk proteins by MP, C – SPT: average wheal diameter upon test with: saline (-), histamine (+), pepsin peptides (PP), commercial milk protein extract (milk) and milk-derived peptides (MP). \* $p < 0.05$ , \*\* $p < 0.005$ , \*\*\* $p < 0.001$ .



Detection of aggregation: A – SEC profiles of PP (A1) and MP (A2), B – tricine electrophoresis, C – 4-20% SDS-PAGE. P – pepsin autoprolysis sample stopped after 60 min, M – milk digestion sample stopped after 60 min, MP – milk-derived peptides <3 kDa, PP – pepsin autoprolysis peptides <3 kDa.

## ACKNOWLEDGEMENTS

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